FORM PTO-1390 * U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 43888-098

U.S. APPLIC. NO. (if known, see 37 CFR 1.5): 09/807692

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/06853	October 2, 2000	October 5, 1999

TITLE OF INVENTION

GLUCOSE SENSOR

APPLICANT(S) FOR DO/EO/US

Motokazu WATANABE, Keiko YUGAWA, Toshihiko YOSHIOKA, Shiro NANKAI, Junko NAKAYAMA, Shoji MIYAZAKI, and Hideyuki BABA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendment has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information.

1. International Search Report by Japanese Patent Office
2. Form PCT/IB/301
3. Form PCT/IB/304

U.S. APPLIC. NO. (if known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/807692	PCT/JP00/06853	43888-098

	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	11 -20 =	0	x $18.00	$	
Independent Claims	1 -3 =	0	x $80.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
			TOTAL OF ABOVE CALCULATIONS =	$ 860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
			TOTAL NATIONAL FEE =	$ 860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 40.00	
			TOTAL FEES ENCLOSED =	$ 900.00	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 500417 in the amount of $ 900.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 500417. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

McDERMOTT, WILL & EMERY
600 13th Street, N.W.
Washington, DC 20005-3096
(202) 756-8000
Facsimile (202) 756-8087

SIGNATURE

Michael E. Fogarty
NAME

36,139
REGISTRATION NUMBER

April 17, 2001
DATE

BASED ON FORM PTO-1390 (Rev. 5-93)

Docket No.: 43888-098

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
	:	
Motokazu WATANABE, et al.	:	
	:	
Serial No.:	:	Group Art Unit:
	:	
Filed: April 17, 2001	:	Examiner:
	:	

For: GLUCOSE SENSOR

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination of the above-referenced application, please amend the application as follows:

IN THE CLAIMS:

Claim 3, line 1, delete " or 2".

Claim 4, lines 1 through 2, change "any one of claims 1 through 3" to --claim 1--.

Claim 5, lines 1 through 2, change "any one of claims 1 through 4" to --claim 1--.

Please add new claims 6 through 11 as follows:

--6. The glucose sensor as set forth in claim 2, wherein said reaction layer further contains calcium ions.

7. The glucose sensor as set forth in claim 2, wherein said salt of gluconic acid is potassium gluconate, sodium gluconate, calcium gluconate, cobalt gluconate, or copper gluconate.

8. The glucose sensor as set forth in claim 3, wherein said salt of gluconic acid is potassium gluconate, sodium gluconate, calcium gluconate, cobalt gluconate, or copper gluconate.

9. The glucose sensor as set forth in claim 2, wherein said reaction layer further contains an electron mediator.

10. The glucose sensor as set forth in claim 3, wherein said reaction layer further contains an electron mediator.

11. The glucose sensor as set forth in claim 4, wherein said reaction layer further contains an electron mediator.--

REMARKS

The above-referenced application is amended to delete the multiple dependency of claims 3, 4, and 5 to avoid the multiple dependent claim filing fee. Attached herewith is an annex which includes clean copies of claims 3, 4, and 5 as amended.

Respectfully submitted,

MCDERMOTT, WILL & EMERY

Michael E. Fogarty
Registration No. 36,139

600 13th Street, N.W.
Washington, DC 20005-3096
(202) 756-8000 MEF:dtb
Date: April 17, 2001
Facsimile: (202) 756-8087

Annex with Claims 3, 4, and 5 as Amended:

3. The glucose sensor as set forth in claim 1, wherein said reaction layer further contains calcium ions.

4. The glucose sensor as set forth in claim 1, wherein said salt of gluconic acid is potassium gluconate, sodium gluconate, calcium gluconate, cobalt gluconate, or copper gluconate.

5. The glucose sensor as set forth in claim 1, wherein said reaction layer further contains an electron mediator.

DESCRIPTION

GLUCOSE SENSOR

Technical Field

The present invention relates to a glucose sensor capable of rapidly and simply quantifying a specific component in a sample with high accuracy. More specifically, the present invention relates to a glucose sensor using pyrrolo-quinoline quinone dependent glucose dehydrogenase.

Background Art

Conventionally, a variety of biosensors have been proposed as a system for simply quantifying a specific component in a sample solution without diluting or stirring the sample solution. As one example of the biosensors, for instance, the following sensor has been known (Japanese Laid-Open Patent Publication No. Hei 2-062952).

This biosensor is fabricated by forming an electrode system comprising a working electrode, a counter electrode and a reference electrode on an electrically insulating base plate by screen printing or other method and forming thereon an enzyme reaction layer comprising a hydrophilic polymer, an oxidoreductase and an electron acceptor in contact with the electrode system.

When a sample solution containing a substrate is dropped on the enzyme reaction layer of this biosensor, the enzyme reaction layer is dissolved, and the substrate and the enzyme react with each other, thereby reducing the electron acceptor. Thereafter, the reduced electron acceptor is electrochemically oxidized, and the concentration of the substrate in the sample solution can be determined from an oxidation current value obtained in this oxidation.

According to the biosensor as mentioned above, in theory, it is possible to measure various substances by selecting an enzyme whose substrate is a substance to be measured.

For instance, if glucose oxidase is selected as the enzyme, it is possible to fabricate a glucose sensor for measuring the concentration of glucose in a sample solution.

In the biosensor having the structure as mentioned above, the enzyme is normally retained in the sensor in a dried state. Since the enzyme is composed mainly of protein, if the enzyme is exposed to moisture in the air, etc. over a long period, there is a risk of the denaturation of the enzyme. Moreover, in an extreme case, there is a risk of the inactivation of the enzyme.

For this reason, if the sensor is stored for a long time, the enzyme activity is lowered and the amount of enzyme that reacts with the substrate becomes insufficient,

and thus there is a possibility that the resultant response current value is not proportional to the concentration of the substrate.

Therefore, in order to obtain a biosensor excelling in the storage stability, it is important to provide an environment for retaining the activity of the enzyme for a long time in the vicinity of the enzyme. Moreover, it is necessary to improve the response of the sensor by facilitating smooth movement of the electrons and substrate during an enzyme reaction.

On the other hand, in order to fabricate a high-performance glucose sensor, pyrrolo-quinoline quinone dependent glucose dehydrogenase (hereinafter referred to as the "PQQ-GDH") is used as the enzyme. In the glucose sensor using the PQQ-GDH, since oxygen is not involved in the catalytic reaction of the PQQ-GDH, this sensor has a characteristic that the enzyme reaction does not receive any effect of dissolved oxygen in blood, etc. Therefore, the measurement value given by this glucose sensor never varies depending on the oxygen partial pressure in the sample solution. In other words, it is possible to obtain a high-performance sensor.

However, in the case where the PQQ-GDH is used as the enzyme of the glucose sensor, it has been revealed that there is a problem that the response value is lowered by storage. This means that the response value is lowered as

the period of storage of the glucose sensor is longer. It is impossible to always use the sensor at a certain time after the fabrication of the sensor. Hence, with a sensor whose response value will be lowered by storage, it is impossible to accurately quantify the concentration of glucose.

In view of such problems, it is an object of the present invention to provide a high-performance glucose sensor having excellent storage stability and an improved response characteristic in an initial stage.

Disclosure of Invention

A glucose sensor according to the present invention is a glucose sensor comprising an electrically insulating base plate; an electrode system including at least a working electrode and a counter electrode formed on the base plate; and a reaction layer containing at least pyrrolo-quinoline quinone dependent glucose dehydrogenase, formed in contact with or in the vicinity of the electrode system, and is characterized in that the reaction layer contains at least one kind of additive selected from the group consisting of gluconic acid and salts of gluconic acid.

It is preferred that the reaction layer further contains at least one kind of additive selected from the group consisting of phthalic acid, salts of phthalic acid,

maleic acid, salts of maleic acid, succinic acid and salts of succinic acid.

It is preferred that the reaction layer further contains calcium ions.

It is preferred that the salt of gluconic acid is potassium gluconate, sodium gluconate, calcium gluconate, cobalt gluconate, or copper gluconate.

It is preferred that the reaction layer further contains an electron mediator.

Brief Description of Drawings

FIG. 1 is a perspective view of a glucose sensor according to one example of the present invention, omitting a reaction layer.

FIG. 2 is a vertical cross-sectional view of the vital part of the glucose sensor shown in FIG. 1.

FIG. 3 is a graph showing the response characteristics of a glucose sensor of Comparative Example 1.

FIG. 4 is a graph showing the response characteristics of a glucose sensor of Example 1 of the present invention.

FIG. 5 is a graph showing the response characteristics of the glucose sensor of Example 1 and glucose sensor of Comparative Example 1 before storage.

FIG. 6 is a graph showing the response

characteristics of a glucose sensor of Example 2 of the present invention.

FIG. 7 is a graph showing the response characteristics of the glucose sensor of Example 2 and glucose sensor of Comparative Example 1 before storage.

FIG. 8 is a graph showing the response characteristics of a glucose sensor of Example 3 of the present invention.

FIG. 9 is a graph showing the response characteristics of the glucose sensor of Example 3 and glucose sensor of Comparative Example 1 before storage.

FIG. 10 is a graph showing the response characteristics of a glucose sensor of Example 4 of the present invention.

FIG. 11 is a graph showing the response characteristics of the glucose sensor of Example 4 and glucose sensor of Comparative Example 1 before storage.

FIG. 12 is a graph showing the response characteristics of a glucose sensor of Example 5 of the present invention.

Best Mode for Carrying Out the Invention

As described above, a glucose sensor of the present invention is obtained by adding gluconic acid and/or a salt thereof to a reaction layer containing the PQQ-GDH as an enzyme.

The present inventors have found that the storage stability of the sensor can be significantly improved by adding gluconic acid and/or a salt thereof to the reaction layer containing the PQQ-GDH. It is deemed that gluconic acid and/or the salt thereof protects the PQQ-GDH from changes in the environment such as the conditions of temperature, humidity and charge, thereby improving the storage stability. In order to enhance such an effect, it is preferred to form the reaction layer by a method in which a mixed solution of gluconic acid and/or a salt thereof and the PQQ-GDH is dropped to a place where the reaction layer is to be formed and then dried. When the reaction layer is formed according to this method, since the enzyme is surrounded by gluconic acid at a molecular level, it is possible to effectively protect the PQQ-GDH from changes in the environment such as the conditions of temperature, humidity and charge. As a result, the activity of the enzyme can be stabilized for a long time.

The present inventors have further found that the response characteristic of the sensor before storage, i.e., the initial characteristic, is improved by adding gluconic acid and/or a salt thereof to the reaction layer containing the PQQ-GDH. Since gluconic acid or the salt thereof is easily dissolved in water, if it is contained in the reaction layer, when a sample solution is added to the reaction layer, the reaction layer is immediately dissolved

in the sample solution, and thus the enzyme reaction and the electrode reaction can proceed smoothly, which is advantageous.

Examples of additives which are expected to produce these effects include potassium gluconate, sodium gluconate, calcium gluconate, cobalt gluconate and copper gluconate as well as gluconic acid. In particular, when potassium gluconate is used, it is possible to obtain a glucose sensor having excellent storage stability and response characteristic and a very low blank value. Here, the blank value is a sensor response value obtained by the use of a sample solution containing no glucose as a substrate, for example, water.

Although phthalic acid, maleic acid, succinic acid and the salts thereof are not as good as gluconic acid and salts of gluconic acid when used alone, since they have an effect of protecting the PQQ-GDH, if they are added together with gluconic acid or a slat thereof, it is possible to further improve the storage stability of the sensor by the synergistic effect. Besides, since phthalic acid, maleic acid, succinic acid and the salts thereof are easily dissolved in water, if they are contained in the reaction layer, when the sample solution is added to the reaction layer, the reaction layer is immediately dissolved in the sample solution and the enzyme reaction and electrode reaction can proceed smoothly, thereby improving

the initial characteristic.

Phthalic acid, maleic acid, succinic acid and the salts thereof are all compounds that can be used as a buffer, and may be added to the reagent for forming the reaction layer by adjusting them to a predetermined pH with acid such as hydrochloric acid and acetic acid or alkali such as NaOH and KOH, if necessary. A suitable pH is between 5.0 and 8.5. Of course, compounds obtained by adding these additives to other buffer may be used.

Since gluconic acid, phthalic acid, maleic acid, succinic acid and the salts thereof are compounds that easily absorb moisture, they should be added during the fabrication of a glucose sensor so that they first come into contact with the enzyme during the fabrication of the glucose sensor, instead of adding them to the enzyme in advance. It is preferred that glucose sensors containing these additives are stored in a sealed state. When storing the glucose sensor, it is preferred to store it in a sealed container containing therein a moisture absorbent such as silica gel.

In a disposable type sensor for measuring 0.5 to 5 μl blood as a sample solution, for the amount of enzyme of 0.2 to 20 U/sensor, the amount of gluconic acid or a salt thereof should be within a range of 1.5 to 150 μg/sensor, and is preferably between 15 and 50 μg/sensor from the viewpoint of the storage stability and a reduction of the

blank value. Meanwhile, for the above-mentioned sensor, the amount of phthalic acid, maleic acid, succinic acid and the salts thereof to be added is preferably between 0.025 and 25 μg/sensor, and more preferably between 0.1 and 3 μg/sensor. Here, U represents unit.

An example of other preferable additive is calcium chloride that gives calcium ions. In general, calcium ions are necessary when the PQQ-GDH forms a dimer. Therefore, when calcium ions are introduced into the reagent for forming the reaction layer by calcium chloride, etc., it is possible to prevent dissociation of the PQQ-GDH to a dimer during or after the fabrication of the sensor, and therefore the calcium ions are useful for retaining the activity of PQQ-GDH. The amount of calcium chloride to be added with respect to the above-mentioned sensor is preferably between 5 and 70 ng (nanogram)/sensor.

It is preferred that the reaction layer of the biosensor of the present invention contains an electron mediator which is reduced with the enzyme reaction. For this electron mediator, it is possible to use potassium ferricyanide, p-benzoquinone and derivatives thereof, phenazine methosulphate, methylene blue, ferrocene and derivatives thereof.

The reaction layer of the biosensor of the present invention may contain a hydrophilic polymer. By adding a hydrophilic polymer to the reaction layer, it is possible

to prevent separation of the reaction layer from the electrode system surface or the base plate surface. Moreover, since the hydrophilic polymer has the effect of preventing cracks in the reaction layer surface, it is effective for an increase of the reliability of the biosensor.

As such a hydrophilic polymer, it is possible to suitably use carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, ethyl hydroxyethyl cellulose, carboxyethyl cellulose, polyvinyl pyrrolidone, polyvinyl alcohol, polyamino acid such as polylysine, polystyrene sulphonate, gelatin and derivatives thereof, polymers of acrylic acid and salts thereof, polymers of methacrylic acid and salts thereof, starch and derivatives thereof, polymers of maleic anhydride and salts thereof, agarose gel and derivatives thereof.

The reaction layer in the biosensor may be placed at various positions as well as on the electrode system formed on the electrically insulating base plate if it does not impair the effects of the present invention. For example, it is possible to place the reaction layer at a position other than on the electrode system of the base plate. Moreover, the biosensor preferably includes a cover member. This cover member is combined with the base plate to form a sample solution supply path between the cover member and

the base plate, for supplying the sample solution to the electrode system. It is possible to position the reaction layer on this cover member's face exposed to the sample solution supply path.

As the method for measuring a current for oxidizing the electron mediator reduced with the enzyme reaction, there are two types of methods: a two-electrode method using only a working electrode and a counter electrode; and a three-electrode method further comprising a reference electrode, and the three-electrode method enables more accurate measurement.

Here, for the reaction layer of the biosensor of the present invention, in addition to the above-mentioned additives, it is possible to add other stabilizer unless it impairs the effects of the present invention. Examples of such a stabilizer include metallic salts, proteins, amino acids, sugars, organic acids, and surface active agents.

Examples of metallic salt include halides such as strontium and manganese, the sulfates and nitrites thereof. Preferred proteins are ones that do not affect the enzyme activity, and examples of such proteins include bovine serum albumin (BSA), egg albumin, and gelatin.

As the amino acid, it is possible to use glycylglycine, polylysine, etc. as well as typical amino acids such as lysine, histidine and glutamic acid. Among them, highly water-soluble amino acids are preferable.

As the sugar, it is possible to use any kinds of sugars, such as monosaccharide, disaccharide, oligosaccharide and polysaccharide. It is also possible to use their derivatives. More specifically, examples of sugars include glucose, fructose, galactose, mannose, xylose, sucrose, lactose, maltose, trehalose, maltotriose, maltocylcyclodextrin, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, dextrin, amylose, glycogen, starch, inulin, glucosamine, inositol, mannitol, sorbitol, ribitol and deoxyglucose.

Examples of the organic acid include α-ketoglutaric acid, malic acid, fumaric acid, cholic acid, and deoxycholic acid.

As the surface active agent, it is preferred to use a nonionic surface active agent.

In addition, boric acid, borax, potassium chloride, sodium chloride, ammonium sulfate, glycerol, Ficoll, EDTA, EGTA, DTT, DTE, GSH, 2-mercaptoethanol, etc. may be added.

The amount of these stabilizers to be added is preferably between 0.01 and 100 parts by weight based on 100 parts by weight of the PQQ-GDH.

In order to prevent pyrrolo-quinoline quinone (PQQ) as a coenzyme from being separated from the PQQ-GDH, PQQ may be added to the reaction layer. The amount of PQQ to be added is preferably between 0.04 and 20 ng/sensor.

As the enzyme PQQ-GDH for use in the present

invention, it is possible to use PQQ-GDH from any source.

The glucose sensor using the PQQ-GDH of the present invention that contains the above-mentioned additives and further contains the above-mentioned stabilizers, if necessary, can retain its performance at low costs without viciously affecting the basic performance of the enzyme.

Some examples will be used to explain the present invention, but the present invention is not necessarily limited to only these examples.

FIG. 1 is an exploded perspective view of a biosensor according to one example of the present invention, omitting the reaction layer. A silver paste is printed on an electrically insulating base plate 1 made of polyethylene terephthalate by screen printing to form leads 2 and 3. Subsequently, a conductive carbon paste containing a resin binder is printed on the base plate 1 to form a working electrode 4. This working electrode 4 is in contact with the lead 2. Further, an insulating paste is printed on the base plate 1 to form an insulating layer 6. The insulating layer 6 covers the peripheral portion of the working electrode 4, so that the area of the exposed portion of the working electrode 4 is kept constant. Next, a ring-shaped counter electrode 5 is formed by printing a conductive carbon paste containing a resin binder on the base plate 1 so as to be in contact with the lead 3.

After forming a reaction layer on the insulating base

plate 1 in a later-described manner, a spacer 8 including a slit 10 and a cover 9 having an air bent 11 are adhered to each other in a positional relationship as shown by the dashed lines of FIG. 1, thereby fabricating the biosensor. A sample solution supply path is formed in the portion of the slit 10 of the spacer 8. The open end of the slit 10 at an end portion of the sensor serves as the sample supply port to the sample solution supply path.

FIG. 2 is a vertical cross sectional view of the biosensor of the present invention. A reaction layer 7 containing an enzyme and an electron mediator is formed on the base plate 1 on which the electrode system is formed. The reaction layer 7 is preferably formed on the electrode system, but it may be formed in the vicinity of the electrode system, for example, on the cover side so that it is exposed to the sample solution supply path. In the illustrated example, the reaction layer 7 is composed of a hydrophilic polymer layer 7a and a layer 7b which contains the PQQ-GDH and additives and is formed on the hydrophilic polymer layer 7a.

Comparative Example 1

5 μl of a 0.5 wt% aqueous solution of sodium salt of carboxymethyl cellulose (hereinafter abbreviated to "CMC") as a hydrophilic polymer was dropped onto the electrode system of the base plate 1 of FIG. 1 and dried in a 50℃

hot-air drier for 10 minutes to form a CMC layer 7a. Subsequently, 5 µl of a mixed aqueous solution containing 1000 U/ml of PQQ-GDH and 50 mM of potassium ferricyanide was dropped onto the CMC layer 7a and dried to form a layer 7b. A glucose sensor was fabricated in such a manner.

Next, as a sample solution, blood conditioned to have a glucose concentration of 30 to 620 mg/dl was prepared. Then, this sample solution was dropped onto the reaction layer 7. When the sample solution containing glucose is supplied to the reaction layer, glucose in the sample is oxidized by the PQQ-GDH. Then, at the same time as the oxidation, potassium ferricyanide in the reaction layer is reduced to potassium ferrocyanide. Here, 30 seconds after the dropping of the sample solution, a voltage of +0.5 V was applied to the working electrode 4 on the basis of the counter electrode 5 so as to oxidize potassium ferrocyanide. Then, 5 seconds later, the value of a current flowing across the counter electrode and the working electrode was measured.

The current value was measured for blood conditioned for a variety of glucose concentrations, and the response characteristic graph of the sensor was produced by plotting the glucose concentration in the horizontal axis and the current value in the vertical axis. The results are shown by the solid line in FIG. 3.

A biosensor fabricated in the same manner was placed

in a sealed container containing silica gel as a moisture absorbent and stored for one week at 40℃, and then the response characteristic graph of this biosensor was produced. The results are shown by the dotted line in FIG. 3.

It would be understood from FIG. 3 that there is a certain correlation between the glucose concentration and the response current value. However, it would be understood that the response characteristic of the sensor stored for one week at 40℃ was lowered in comparison with the sensor immediately after the fabrication, i.e., before storage.

Example 1

After forming the CMC layer 7a in the same manner as in Comparative Example 1, 5 μl of a mixed aqueous solution containing 1000 U/ml of PQQ-GDH, 50 mM of potassium ferricyanide and 40 mM of potassium gluconate was dropped onto the CMC layer 7a and dried to form the layer 7b. A glucose sensor was fabricated in such a manner.

Next, in the same manner as in Comparative Example 1, the response characteristic graph was produced for the sensor immediately after the fabrication and the sensor after being stored in a sealed container containing silica gel for one week at 40℃. The results are shown in FIG. 4. It would be understood from FIG. 4 that there is a certain

correlation between the glucose concentration and the response current value. It would be understood by a comparison with Comparative Example 1 that the sensor of this example had a smaller lowering in the response current value in the response after the one-week storage at 40℃, particularly in a range of not less than 400 mg/dl. Hence, it would be understood that the storage characteristic of the glucose sensor is significantly improved by the addition of potassium gluconate.

FIG. 5 shows a comparison of the response characteristics before storage between the sensor of Comparative Example 1 containing no potassium gluconate and the sensor of this example containing potassium gluconate. It is seen from FIG. 5 that the glucose sensor containing potassium gluconate has higher response values in the vicinity of 600 mg/dl than the glucose sensor containing no potassium gluconate. Hence, it would be understood that it is possible to improve the response characteristic of the glucose sensor in a high-concentration range by the addition of potassium gluconate.

Example 2

After forming the CMC layer 7a in the same manner as in Comparative Example 1, 5 μl of a mixed aqueous solution containing 1000 U/ml of PQQ-GDH, 50 mM of potassium ferricyanide, 40 mM of potassium gluconate and 0.5 mM of

potassium hydrogen phthalate was dropped onto the CMC layer 7a and dried to form the layer 7b. A glucose sensor was fabricated in such a manner.

In the same manner as in Comparative Example 1, the response characteristic graph was produced for the sensor immediately after the fabrication and the sensor after being stored in a sealed container containing silica gel for one week at 40℃. The results are shown in FIG. 6. It would be understood from FIG. 6 that there is almost no difference in the response characteristics between the sensor immediately after the fabrication and the sensor after the one week storage at 40℃, and the storage characteristic of the sensor of this example is significantly improved in comparison with Comparative Example 1.

FIG. 7 shows a comparison of the response characteristics before storage between the sensor of Comparative Example 1 containing no potassium gluconate and the sensor of this example containing potassium gluconate and potassium hydrogen phthalate. It is seen from FIG. 7 that the glucose sensor containing potassium gluconate and potassium hydrogen phthalate has higher response values in the vicinity of 600 mg/dl compared with the glucose sensor of Comparative Example 1. Hence, it would be understood that it is possible to improve the response characteristic of the glucose sensor in a high-concentration range by the

addition of potassium gluconate and potassium hydrogen phthalate.

Example 3

After forming the CMC layer 7a in the same manner as in Comparative Example 1, 5 µl of a mixed aqueous solution containing 1000 U/ml of PQQ-GDH, 50 mM of potassium ferricyanide, 40 mM of potassium gluconate and 0.5 mM of maleic acid was dropped onto the CMC layer 7a and dried to form the layer 7b. A glucose sensor was fabricated in such a manner.

In the same manner as in Comparative Example 1, the response characteristic graph was produced for the sensor immediately after the fabrication and the sensor after being stored in a sealed container containing silica gel for one week at 40℃. The results are shown in FIG. 8. It would be understood from FIG. 8 that there is almost no difference in the response characteristics between the sensor immediately after the fabrication and the sensor after the one-week storage at 40℃, and the storage characteristic of the sensor of this example is improved in comparison with Comparative Example 1.

FIG. 9 shows a comparison of the response characteristics before storage between the sensor of Comparative Example 1 containing no potassium gluconate and the sensor of this example containing potassium gluconate

and maleic acid. It is seen from FIG. 9 that the glucose sensor containing potassium gluconate and maleic acid has higher response values in the vicinity of 600 mg/dl. Hence, it would be understood that it is possible to improve the response characteristic of the glucose sensor in a high-concentration range by the addition of potassium gluconate and maleic acid.

Example 4

After forming the CMC layer 7a in the same manner as in Comparative Example 1, 5 µl of a mixed aqueous solution containing 1000 U/ml of PQQ-GDH, 50 mM of potassium ferricyanide, 40 mM of potassium gluconate and 0.5 mM of succinic acid was dropped onto the CMC layer 7a and dried to form the layer 7b. A glucose sensor was fabricated in such a manner.

In the same manner as in Comparative Example 1, the response characteristic graph was produced for the sensor immediately after the fabrication and the sensor after being stored in a sealed container containing silica gel for one week at 40℃. The results are shown in FIG. 10. It would be understood from FIG. 10 that there is almost no difference in the response characteristics between the sensor immediately after the fabrication and the sensor after the one-week storage at 40℃, and the storage characteristic of the sensor of this example is improved in

comparison with Comparative Example 1.

FIG. 11 shows a comparison of the response characteristics before storage between the sensor of Comparative Example 1 and the sensor of this example containing potassium gluconate and succinic acid. It is seen from FIG. 11 that the glucose sensor containing potassium gluconate and succinic acid has higher response values in the vicinity of 600 mg/dl. Hence, it would be understood that it is possible to improve the response characteristic of the glucose sensor in a high-concentration range by the addition of potassium gluconate and succinic acid.

Example 5

After forming the CMC layer 7a in the same manner as in Comparative Example 1, 5 μl of a mixed aqueous solution containing 1000 U/ml of PQQ-GDH, 50 mM of potassium ferricyanide, 40 mM of potassium gluconate, 0.5 mM of potassium hydrogen phthalate and 75 μM of calcium chloride was dropped onto the CMC layer 7a and dried to form the layer 7b. A glucose sensor was fabricated in such a manner.

In the same manner as in Comparative Example 1, the response characteristic graph was produced for the sensor immediately after the fabrication and the sensor after being stored in a sealed container containing silica gel for one week at 40℃. The results are shown in FIG. 12. It

would be understood from FIG. 12 that there is almost no difference in the response characteristics between the sensor immediately after the fabrication and the sensor after the one-week storage at 45℃, and the storage characteristic of the sensor of this example is superior under a high-temperature storage condition, that is, one-week storage at 45℃.

Industrial Applicability

According to the present invention, as described above, it is possible to obtain a high-performance glucose sensor having excellent storage stability and an improved response characteristic.

CLAIMS

1. A glucose sensor comprising: an electrically insulating base plate; an electrode system including at least a working electrode and a counter electrode formed on said base plate; and a reaction layer containing at least pyrrolo-quinoline quinone dependent glucose dehydrogenase, formed in contact with or in the vicinity of said electrode system, wherein said reaction layer contains at least one kind of additive selected from the group consisting of gluconic acid and salts thereof.

2. The glucose sensor as set forth in claim 1, wherein said reaction layer further contains at least one kind of additive selected from the group consisting of phthalic acid, salts of phthalic acid, maleic acid, salts of maleic acid, succinic acid and salts of succinic acid.

3. The glucose sensor as set forth in claim 1 or 2, wherein said reaction layer further contains calcium ions.

4. The glucose sensor as set forth in any one of claims 1 through 3, wherein said salt of gluconic acid is potassium gluconate, sodium gluconate, calcium gluconate, cobalt gluconate, or copper gluconate.

5. The glucose sensor as set forth in any one of claims 1 through 4, wherein said reaction layer further contains an electron mediator.

ABSTRACT

The present invention provides a high-performance glucose sensor having excellent storage stability and an improved response characteristic. This sensor comprises: an electrically insulating base plate; an electrode system including at least a working electrode and a counter electrode formed on the base plate; and a reaction layer containing at least pyrrolo-quinoline quinone dependent glucose dehydrogenase, formed in contact with or in the vicinity of the electrode system, and the reaction layer contains at least one kind of additive selected from the group consisting of gluconic acid and salts thereof.

FIG.1

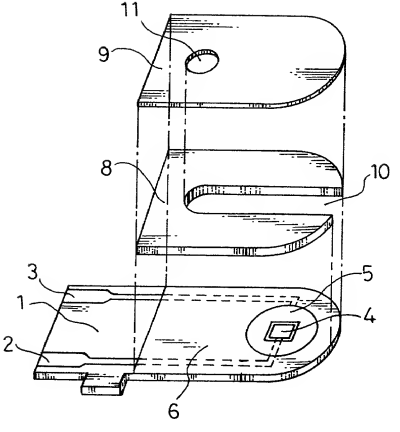


FIG. 2

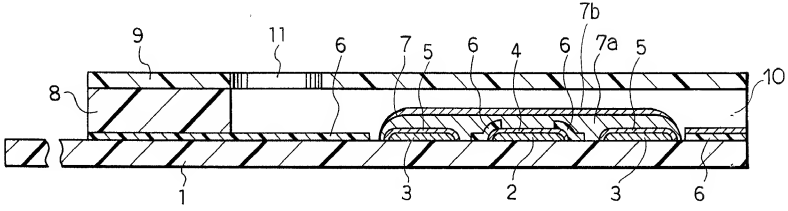


FIG. 3

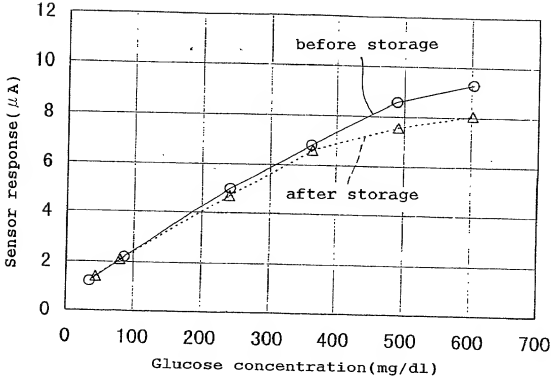

12
10
8
6
4
2
0
Sensor response(μA)
before storage
after storage
0
100
200
300
400
500
600
700
Glucose concentration(mg/dl)

FIG. 4

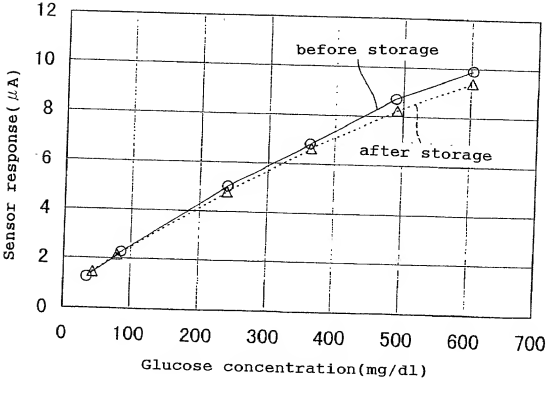

12
10
8
6
4
2
0
Sensor response(μA)
before storage
after storage
0
100
200
300
400
500
600
700
Glucose concentration(mg/dl)

FIG.5

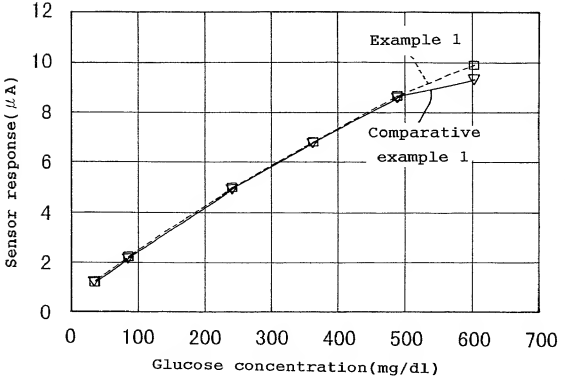

Example 1
Comparative example 1
Sensor response(μA)
Glucose concentration(mg/dl)
0
2
4
6
8
10
12
0
100
200
300
400
500
600
700

FIG.6

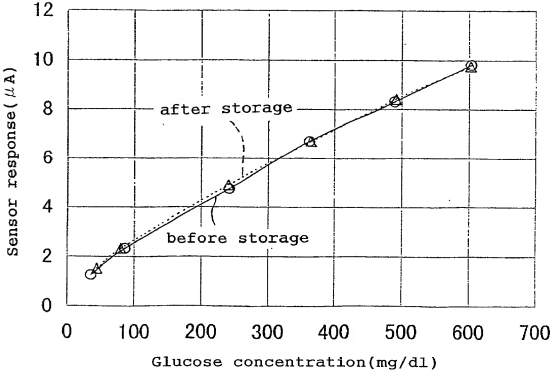

after storage
before storage
Sensor response(μA)
Glucose concentration(mg/dl)
0
2
4
6
8
10
12
0
100
200
300
400
500
600
700

FIG.7

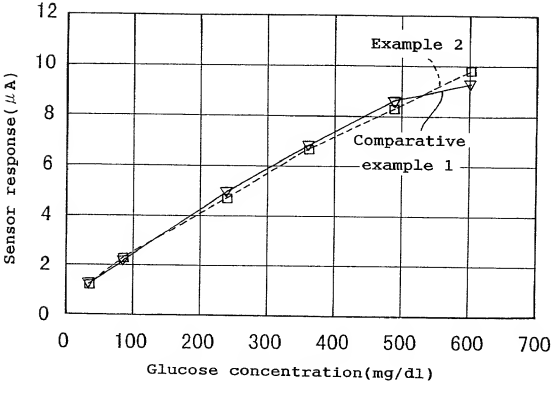

12
10
8
6
4
2
0
Sensor response(μA)
0
100
200
300
400
500
600
700
Glucose concentration(mg/dl)
Example 2
Comparative
example 1

FIG.8

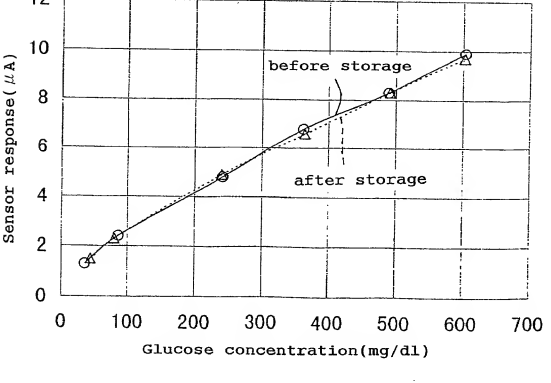

12
10
8
6
4
2
0
Sensor response(μA)
0
100
200
300
400
500
600
700
Glucose concentration(mg/dl)
before storage
after storage

FIG.9

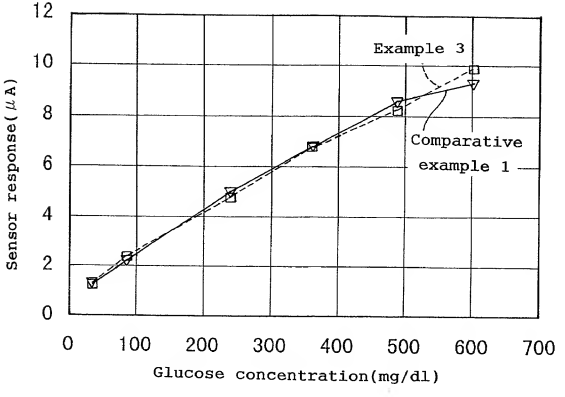

Example 3
Comparative example 1
Sensor response(μA)
Glucose concentration(mg/dl)
0
2
4
6
8
10
12
0
100
200
300
400
500
600
700

FIG.10

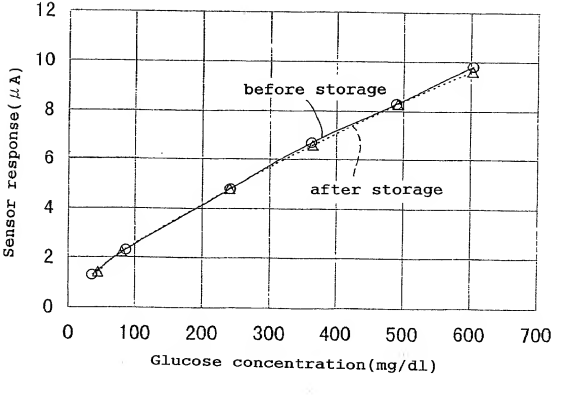

before storage
after storage
Sensor response(μA)
Glucose concentration(mg/dl)
0
2
4
6
8
10
12
0
100
200
300
400
500
600
700

FIG.11

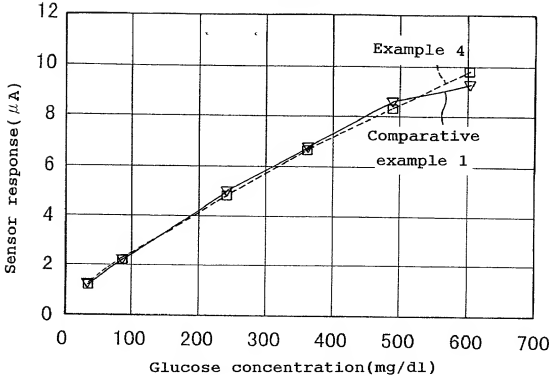
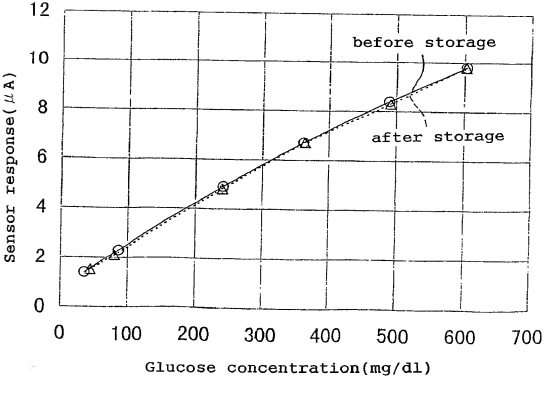

12
10
8
6
4
2
0
Sensor response(μA)
Example 4
Comparative
example 1
0
100
200
300
400
500
600
700
Glucose concentration(mg/dl)

FIG.12


12
10
8
6
4
2
0
Sensor response(μA)
before storage
after storage
0
100
200
300
400
500
600
700
Glucose concentration(mg/dl)

Attorney Docket No.: _____

COMBINED DECLARATION/POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor(s), I (we) hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Glucose sensor, the specification of which

(check one)

____ is attached hereto.

____ was filed on _____ as
United States Application No. _____

__X__ PCT International Patent Application No. PCT/JP00/06853
filed on October 2, 2000
and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

			Priority Claimed	
Hei 11-284871 (Number)	Japan (Country)	October 5, 1999 (Day/Month/Year Filed)	X Yes	____No
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	____Yes	____No
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	____Yes	____No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

PCT/JP00/06853 (Appln. Serial No.)	October 2, 2000 (Filing Date)	pending (Status-patented, pending, abandoned)
_____ (Appln. Serial No.)	_____ (Filing Date)	_____ (Status-patented, pending, abandoned)

I hereby appoint as my attorneys, with full power of substitution and revocation, to prosecute the patent application identified above and to transact all business in the U.S. Patent and Trademark Office connected therewith: Edward A. Becker, Reg. No. 37,777; Stephen A. Becker, Reg. No. 26,527; Marcel K. Bingham, Reg. No. 42,327; John G. Bisbikis, Reg. No. 37,095; Daniel Bucca, Reg. No. 42,368; Kenneth L. Cage, Reg. No. 26,151; Stephen C. Carlson, Reg. No. 39,929; Tom A. Corrado, Reg. No. 42,439; Paul Devinsky, Reg. No. 28,553; Laura A. Donnelly, Reg. No. 38,435; Margaret M. Duncan, Reg. No. 30,879; Brian E. Ferguson, Reg. No. 36,801; Michael F. Fogarty, Reg. No. 36,139; Willem F. Gadiano, Reg. No. 37,136; Keith E. George, Reg. No. 34,111; John A. Hankins, Reg. No. 32,029; Brian D. Hickman, Reg. No. 35,894; Eric J. Kraus, Reg. No. 36,190; Patrick B. Law, Reg. No. 41,549; Robert E. LeBlanc, Reg. No. 17,219; Jack Q. Lever, Reg. No. 28,149; Raphael V. Lupo, Reg. No. 28,363; Christine F. Martin, Reg. No. 39,762; Michael A. Messina, Reg. No. 33,424; Eugene J. Molinelli, Reg. No. 42,901; Christopher J. Palermo, Reg. No. 42,056; Joseph H. Paquin, Jr., Reg. No. 31,647; Robert L. Price, Reg. No. 22,685; Gene Z. Rubinson, Reg. No. 33,351; Joy Ann G. Serauskas, Reg. No. 27,952; David A. Spenard, Reg. No. 37,449; Arthur J. Steiner, Reg. No. 26,106; David L. Stewart, Reg. No. 37,578; Michael D. Switzer, Reg. No. 39,552; Leonid D. Thenor, Reg. No. 39,397; Keith J. Townsend, Reg. No. 40,358; Aaron Weisstuch, Reg. No. P41,557; Edward J. Wise, Reg. No. 34,523; Alexander V. Yampolsky, Reg. No. 36,324; and Robert W. Zelnick, Reg. No. 36,976 all of

40 -

Please address all correspondence and telephone calls to:

MCDERMOTT, WILL & EMERY
600 13th Street, N.W.
WASHINGTON, D.C. 20005-3096
Telephone: 202-756-8000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full name of sole or first inventor Motokazu WATANABE

Inventor's signature Motokazu Watanabe Date February 21, 2001

Residence Katano-shi, Osaka Japan JPX Citizenship Japanese

Post Office Address 4-28-402, Amanogahara, Katano-shi, Osaka 576-0034 Japan

2-00 Full name of second inventor Keiko YUGAWA

Inventor's signature Keiko Yugawa Date February 26, 2001

Residence Nara-shi, Nara Japan JPX Citizenship Japanese

Post Office Address 708-1-513, Gakuendaiwacho 6-chome, Nara-shi, Nara 631-0041 Japan

3-00 Full name of third inventor Toshihiko YOSHIOKA

Inventor's signature Toshihiko Yoshioka Date February 28, 2001

Residence Hirakata-shi, Osaka Japan JPX Citizenship Japanese

Post Office Address 15-15-307, Takatsukacho, Hirakata-shi, Osaka 573-0035 Japan

4-00 Full name of fourth inventor Shiro NANKAI

Inventor's signature Shiro Nankai Date February 28, 2001

Residence Hirakata-shi, Osaka Japan JPX Citizenship Japanese

Post Office Address 4-50-12, Nasuzukuri, Hirakata-shi, Osaka 573-0071 Japan

5-00 Full name of fifth inventor Junko NAKAYAMA

Inventor's signature Junko Nakayama Date February 21, 2001

Residence Onsen-gun, Ehime Japan JPX Citizenship Japanese

Post Office Address 298-1, Oaza-Uemura-Kou, Shigenobucho, Onsen-gun, Ehime 791-0221 Japan

6-00 Full name of sixth inventor Shoji MIYAZAKI

Inventor's signature Shoji Miyazaki Date February 21, 2001

Residence Matsuyama-shi, Ehime Japan JPX Citizenship Japanese

Post Office Address 1052, Minamisayacho, Matsuyama-shi, Ehime 791-8032 Japan

7-00 Full name of seventh inventor Hideyuki BABA

Inventor's signature Hideyuki Baba Date February 21, 2001

Residence Matsuyama-shi, Ehime Japan JPX Citizenship Japanese

Post Office Address 340-2, Kitakumecho, Matsuyama-shi, Ehime 790-0923 Japan